

## Brief Note

# Assessment of gonadal follicle size in the invading bivalve *Limnoperna fortunei* (Mytilidae)

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**ABSTRACT:** *Limnoperna fortunei* is an invasive gonochoristic and byssate freshwater bivalve originary from Southeast Asia. It shows great adaptive-reproductive ability, so knowledge of the gonadal cycle is an important factor for the prevention and control of this bioinvasion. This species is highly damaging to natural and human environments. We analyzed the distribution and maturity state of reproductive follicles in the mantle of both male and females. Male results are not shown but, in general, they followed the same pattern as that of females. Routine histological techniques included serial longitudinal sections and transversal sections in three body regions (anterior, middle and posterior). Oocytes with conspicuous nucleoli were measured on both types of sections to estimate the maturity stage in the different regions. ANOVA indicates that there were no significant differences in maturity ratio between the studied regions, so that a small number of sections would render precise results to assess maturity.

The golden mussel, *Limnoperna fortunei* (Dunker, 1857) (Bivalvia: Mytilidae) is a gonochoristic freshwater invading bivalve with an epibyssate habit, and lives attached to natural and artificial hard substrates (Darrigran, 2002). Its original range is Southeast Asia, from where it invaded Hong Kong, Japan and Taiwan (Darrigran, 2002). It was first recorded along the shores of the Río de la Plata in 1991, at Balneario Bagliardi (34°55'S-57°49'W), Buenos Aires Province, Argentina (Darrigran and Pastorino, 1995). The invasion of South America by the golden mussel was facilitated by its high

adaptive-reproductive capability that enabled a notorious upstream dispersion at a rate of about 240 km/yr into the Plata and Guaíba basins (Mansur *et al.*, 1999; Darrigran, 2002). Thus, during its invading process the species dispersed from a temperate to a subtropical climate. It causes considerable damage in modifying natural environments and causes macrofouling in human environments, obstructing water pumps at industrial plants, hydroelectric power plants, drinking-water plants, irrigation systems, etc. (Darrigran, 2010).

The study and control of bioinvasions in general is important for preservation of natural biodiversity (Darrigran, 2010). *L. fortunei* and *Corbicula fluminea* (Müller, 1774), among the invasive freshwater bivalves in South America, have been particularly successful

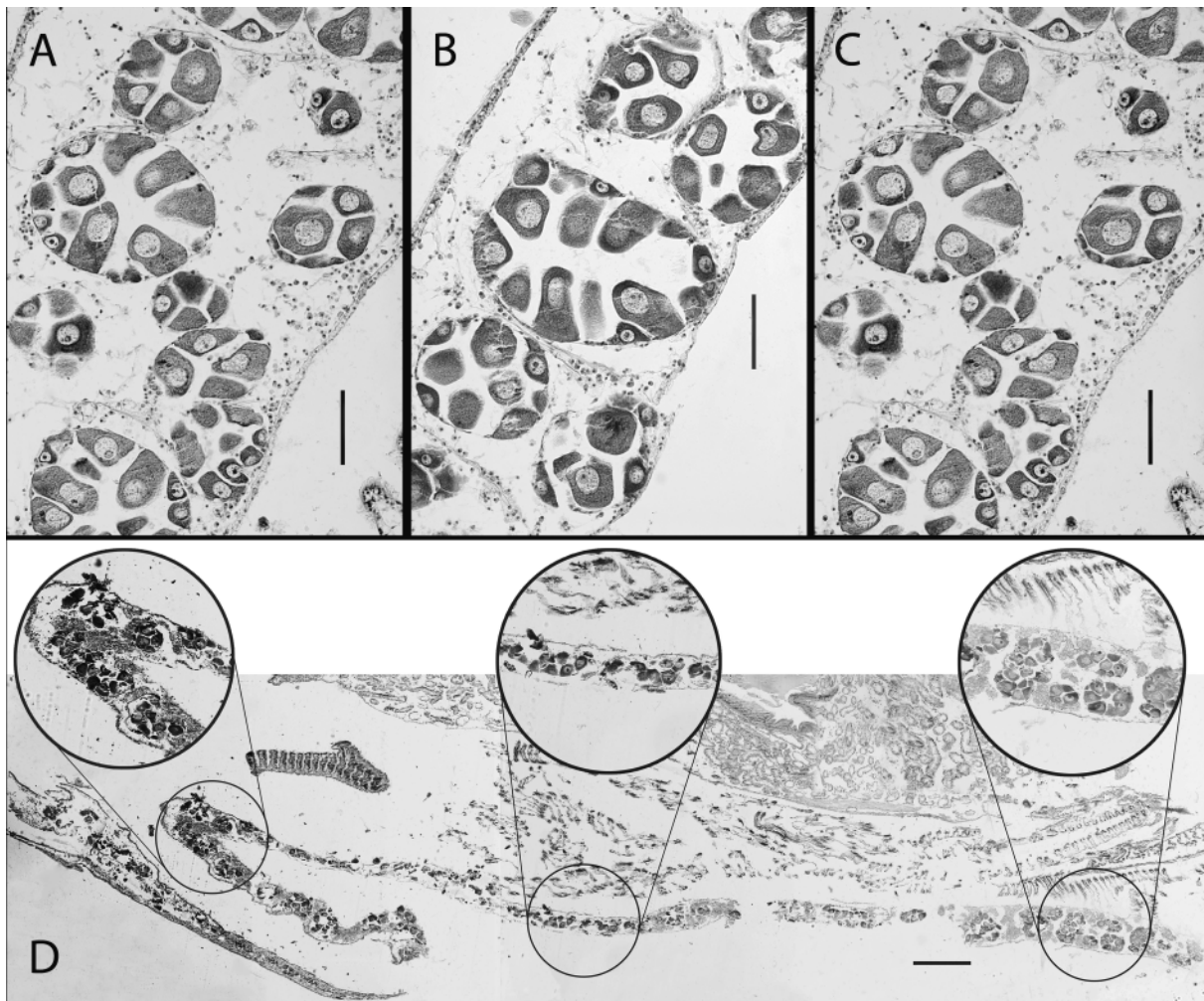
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(Darrigran, 2002). Study of gonadal cycles in invasive species renders important information for the understanding the invasion process (Kolar and Lodge, 2002), and hence for developing of prevention and control techniques at industrial plants (Darrigran *et al.*, 2003).

As in other mytilid species, *e.g.*, *Mytilus edulis* (Linnaeus, 1758), *L. fortunei* has gonadal follicles throughout the mantle and in the visceral mass (Damborenea and Penchaszadeh, 2006). The first gonadal study of this species in South America was carried out on specimens from Balneario Bagliardi one year after they settled there. Such study included transversal histological sections in anterior, middle, and posterior regions of the specimens (Darrigran *et al.*, 1999). Gonadal cycle of this species indicates that it may adapt its reproductive cycle to new environmental requirements.

Herein we analyzed the distribution and maturity stage of the gonadal follicles of *L. fortunei* across the mantle, to determine if their distribution is uniform from the anterior to the posterior region of male and female specimens, as described for *M. edulis* (Lowe *et al.*, 1982).

Sexually mature specimens of both sexes, 12-28 mm long, were collected from a population living in the subtropical climate of Itapua (Praia das Pombas) (30°18' S-51°02' W), Guaíba Basin (Brazil). The specimens were fixed in Bouin, washed in 70° alcohol and the length of valves were measured. Transversal sections (7 µm thick) at anterior, middle, and posterior regions were obtained for each mussel (N=21), and also longitudinal serial sections were obtained from some other 21 mussels. All sections were stained with Mayer's hematoxyline and eosine. Sections were pho-



**FIGURE 1.** Micrographs of female mantle of *Limnoperna fortunei*. Transversal sections of (A) anterior, (B) middle, (C) posterior regions; scale=100 µm. (D) Longitudinal sections; scale=0.5 mm.

tographed and oocytes with conspicuous nucleoli were measured with Axiovision v. 4.4 digital imaging software and photographs were taken using an Axiocam HRc digital camera attached to a Zeiss Axio Imager Z1 microscope. Measurements obtained from longitudinal sections were compared with those from transversal sections. To estimate the existence of significant differences between maturity stages in the three studied regions, ANOVA of oocyte average size in transversal sections and longitudinal sections was performed. Results from males are not shown but, in general, they followed the same pattern as that of females.

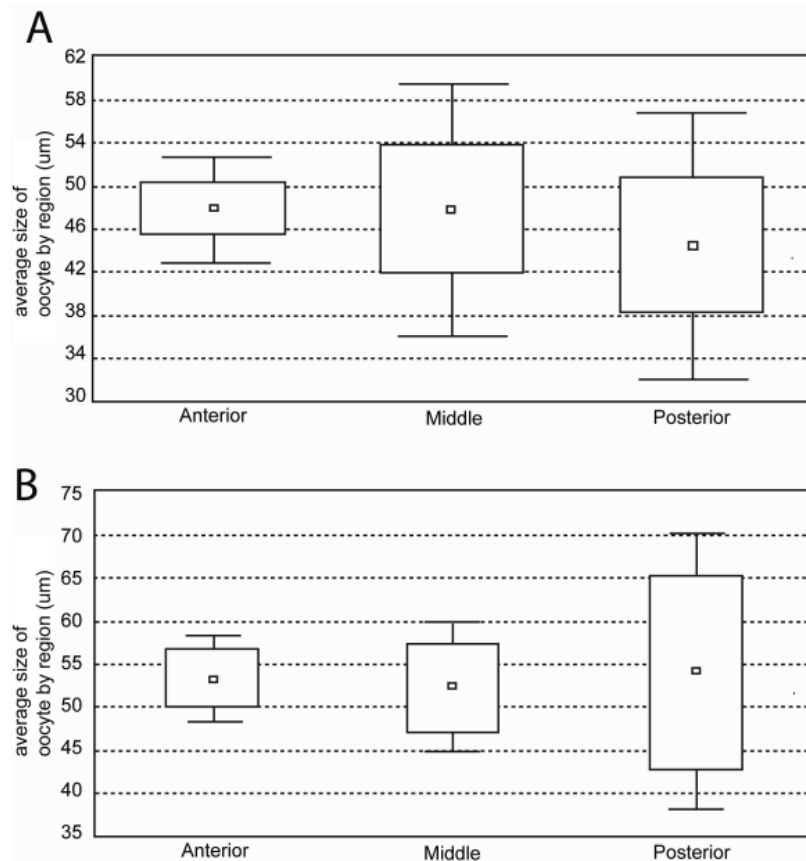
Light microscopy analysis of female transversal sections showed no difference between the distribution of gonadal follicles in the three mantle regions (Fig. 1A-C). Likewise, the analysis of female longitudinal sections revealed a uniform distribution of fol-

licles throughout the mantle (Fig. 1D). Therefore, no significant difference in oocyte size was found between the three studied regions, whether observed in transversal or longitudinal sections (Fig. 2).

Results indicate that there is no need of studying several body regions to assess the gonadal status of male and female golden mussels. This entails that a low number of sections will render precise results with reduced costs.

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**Figure 2.** Comparison of average size of *Limnoperna fortunei* oocytes between the anterior, middle and posterior regions in: (A) transversal sections; (B) longitudinal sections.  $\square$  : arithmetic mean;  $\square$  : 1.00 standard deviation;  $\ddagger$ : 1.96 standard deviation

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